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**Temporal genetic stability in natural populations of the waterflea *Daphnia magna* in response to strong selection pressure**

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## 22    **Abstract**

23    Studies monitoring changes in genetic diversity and composition through time allow a unique  
24    understanding of evolutionary dynamics and persistence of natural populations. However, such  
25    studies are often limited to species with short generation times that can be propagated in the  
26    laboratory or few exceptional cases in the wild. Species that produce dormant stages provide  
27    powerful models for the reconstruction of evolutionary dynamics in the natural environment. A  
28    remaining open question is to what extent dormant egg banks are an unbiased representation of  
29    populations and hence of the species' evolutionary potential, especially in presence of strong  
30    environmental selection. We address this key question using the water flea *Daphnia magna*,  
31    which produces dormant stages that accumulate in biological archives over time. We assess  
32    temporal genetic stability in three biological archives, previously used in resurrection ecology  
33    studies showing adaptive evolutionary responses to rapid environmental change. We show that  
34    neutral genetic diversity does not decline with the age of the population and it is maintained in  
35    presence of strong selection. In addition, by comparing temporal genetic stability in hatched and  
36    unhatched populations from the same biological archive, we show that dormant egg banks can be  
37    consulted to obtain a reliable measure of genetic diversity over time, at least in the multi-decadal  
38    time frame studied here. The stability of neutral genetic diversity through time is likely mediated  
39    by the buffering effect of the resting egg bank.

40

**Introduction**

Understanding how environmental change affects the genetic composition of populations over time is critical for gauging how species respond and persist to environmental change. However, excluding a few exceptional cases of long-term studies (e.g. Galapagos Darwin finches, Grant & Grant 2002) the processes underpinning evolutionary dynamics through time often remain elusive. Because of logistic difficulties associated with accessing temporal samples, changes in genetic composition in response to environmental change are more frequently studied in spatial (synchronic) rather than temporal (allochronic) settings. Most studies analysing temporal dynamics involve experimental evolution in the laboratory or controlled mesocosm experiments (Barrick *et al.* 2009; Blount *et al.* 2012; Kawecki *et al.* 2012) with exceptional studies that reconstruct evolution of natural populations using transplant experiments in the wild (Reznick *et al.* 1997). For species that cannot be easily manipulated experimentally, or for which temporal samples are inaccessible, the ‘space-for-time’ substitution (Fukami & Wardle 2005 ) is frequently adopted as a surrogate to study long-term evolutionary dynamics. Space-for-time analyses assume that two different conditions at two points in space can be treated as though they are in the same region at two different time points. This approach has its limitations as rates of adaptation at different spatial scales can differ compared to temporal variation in the same population evolving in time (Merila & Hendry 2014). It is, however, the only possible approach when temporal samples are not accessible. A powerful alternative that allows studying evolutionary dynamics in natural populations over time is the analysis of genetic change in species producing dormant stages. Zooplankters are among the species that adopt this strategy. In these species, dormant stages are early stage embryos that escape environmental hardships by arresting development to remain dormant and protected from the elements by a resistant capsule.

These dormant stages become buried in the sediment at the bottom of lakes, remaining viable for decades, centuries or more (Frisch *et al.* 2014; Yashina *et al.* 2012) and providing a powerful resource to generate long-term data in natural systems (Bidle *et al.* 2007; Frisch *et al.* 2014; Härnström *et al.* 2011; Orsini *et al.* 2013b). Freshwater crustaceans are a group of organisms for which the practise of ‘resurrection ecology’, the study of individuals and populations hatched from dormant stages retrieved from dated lake sediments (Kerfoot *et al.* 1999), has been widely applied to study micro-evolutionary responses to environmental change, mostly via the analysis of phenotypic traits or genotypic trait values (e.g. Cousyn *et al.* 2001; Decaestecker *et al.* 2007; Frisch *et al.* 2014; Hairston *et al.* 1999; Stoks *et al.* 2016; Weider & Pijanowska 1993). These studies have provided important insights into micro-evolutionary responses to environmental change in natural populations through time. However, the study of temporal changes in genetic diversity has been largely neglected, and the few studies that addressed changes in genetic diversity over time used a small set of genetic markers and thus had limited power (Cousyn *et al.* 2001).

The increasing availability of genetic tools provides great promise for the use of dormant stages extracted from biological archives to study evolutionary responses to environmental change (Orsini *et al.* 2013b). Yet, it has been questioned whether dormant egg banks that are recovered from layered sediments are representative of the genetic diversity and composition of the populations during historical times (Jankowski & Straile 2003), especially in presence of strong selection pressure. It is possible that with increasing age of the sediment non-random mortality of the eggs might result in biased estimates of genetic diversity and composition. With increasing age the hatching success of the dormant stages may be reduced, and as a result genetic assays are often based on relatively small numbers of individuals. Hence, an important methodological

issue to be addressed is the minimum threshold sample size required to represent the genetic diversity of dormant populations.

The analysis of temporal genetic stability in layered dormant egg banks allows a retrospective assessment of environmental impacts on genetic diversity. A key question is whether neutral genetic diversity in natural populations is reduced following strong environmental selection pressure leaving a long-term signature of reduced genetic diversity. Another important aspect is to assess whether genetic drift has a strong impact on the genetic composition of populations, which would result in significant allele frequency differences between years and an overall reduced allelic richness over time, limiting the ability of populations and species to cope with selection pressure.

To address the conceptual and methodological issues outlined above, we analyze changes in temporal genetic diversity of three biological archives containing layered dormant egg banks of *D. magna*, which we know have experienced strong selection pressure. *Daphnia* arguably has one of the best-understood ecologies of any animal, primarily because of its central role in food-webs of inland water habitats and its amenability as ecological model system (Altshuler *et al.* 2011; Miner *et al.* 2012). *D. magna* is a cyclical parthenogenetic zooplankter, with a life cycle alternating between asexual clonal reproduction in favorable environmental conditions and sexual reproduction in the presence of deteriorating environmental conditions. When environmental conditions deteriorate the asexual production of males and the production of sexual eggs is induced. The resulting early stage embryos arrest their development remaining dormant and protected from the environment within a resistant capsule (ephippium) until favorable environmental condition induce hatching.

Using microsatellite (up to 72) and SNP (up to 840) markers, we measure neutral genetic diversity changes over time in the three biological archives mentioned above. By comparing genetic diversity before and after a well-documented and strong environmental shift, we test whether genetic adaptation to a strong environmental change leads to genetic erosion due to severe bottlenecks. If strong selection pressure determines genetic erosion, we expect a consistent decline in genetic diversity in the three archives following environmental selection. A directional decline in genetic diversity from old to recent (sub)populations along a sedimentary archive would indicate loss of genetic diversity through genetic drift. Conversely, if loss of genetic diversity occurs with aging of the dormant (sub)populations-from recent to old layers-, we expect a consistent decline in genetic diversity with age in the three archives. In addition to testing the hypotheses outlined above, we test whether hatched (sub)populations from the dormant egg bank are an unbiased sample of the dormant egg bank by comparing both genetic diversity and composition of the hatched (sub)populations with that of the unhatched egg bank in one of the biological archives. Finally, we determine the threshold sample size and marker set required to assess genetic diversity in *D. magna* populations using a rarefaction analysis on the (sub)populations from the biological archives and on a set of 19 populations with relatively large sample size and previously used in a population genomic study (Orsini *et al.* 2012). With this approach we identify the threshold sample size needed to capture changes in genetic diversity in our study species both in space and over multi-decadal time spans. We also identify the combination of sample sizes and number of markers appropriate to describe neutral genetic diversity in our study species. Our study shows that dormant egg banks can be consulted to obtain a reliable measure of genetic diversity over time, at least in the multi-decadal time frame studied here employing a combination of reasonably small number of markers and sample sizes.

## 133    **Materials and methods**

### 134    *Source material*

135    The material used in this study consisted of three sedimentary archives from which several  
136    (sub)populations of *D. magna* were sampled and of 19 populations isolated from 19 ponds  
137    distributed in the landscape along orthogonal gradients of selection (Table S1) (Orsini *et al.*  
138    2012). Hereafter, we use the term population when referring to populations from the spatial  
139    survey, hence populations in the landscape; we use the term (sub)populations when referring to  
140    temporally sampled populations along the sedimentary archives. The sedimentary archives were  
141    sampled using a piston corer or a Plexiglas tube of 6cm of diameter. After sampling, the cores  
142    were brought to the laboratory where they were sliced in incremental intervals of 0.5 or 1cm.  
143    Each layer was then stored separately in the dark at 4°C. A few grams of sediment were collected  
144    from 10 to 15 depths for radiolabeling and dating using established techniques (Appleby *et al.*  
145    1986). Based on radio dating or using fractions of organic material and assuming constant  
146    sedimentation rates (e.g. Oud Heverlee Zuid; Cousyn *et al.* 2001) the year of each layer and  
147    hence of the dormant eggs isolated from each layer were established. By aligning the chronology  
148    of each core with the known history of the lake where the core was sampled, we could identify  
149    how populations respond to major ecological shifts in the lakes.

150        *Core OH (Oud Heverlee, Belgium) (50°50' 13.12"; 4°39' 48.87"E).* This core was  
151    sampled in 1997 from a shallow artificial pond established in 1970 for fish culture and spans 23  
152    years (Cousyn *et al.* 2001). The material obtained from this biological archive has been used in  
153    one of the first resurrection ecology studies showing adaptive responses of the crustacean *D.*  
154    *magna* to fish predation (Cousyn *et al.* 2001). The same resurrected (sub)populations from this  
155    archive were used more than a decade later to identify genome-wide signatures of selection



induced by vertebrate predators (Orsini *et al.* 2012). During the first three years after its creation, the pond was stocked annually with a low number of benthivorous fish. From 1973 to the early 1980s, a very high biomass of planktivorous fish (300 kg/ha) was stocked each year. The amount of stocked fish subsequently was reduced and varied from the mid-1980s until 1993 when fish stocking ceased. Based on changes in fish predation pressure, we thus differentiate three main periods: before fish stocking (1970–1972; Bottom, low predation); intense fish stocking (1976–1979, Middle; high predation); and after relaxing fish stocking (ca. 1988, Top; relaxed predation; see also (Cousyn *et al.* 2001; Stoks *et al.* 2016). For each of these time periods 12 *D. magna* dormant eggs were hatched following standard protocols (Marcus 1990; Onbe 1978) and clonal lineages established for a total of 36 distinct genotypes.

*Core OM2 (Oude Meren 2, Belgium)* (50°51'51.61", 4°51'48.98"E). This core was obtained in 2006 from a shallow artificial pond in Belgium. Material from this biological archive was used to document host-parasite co-evolutionary dynamics (Decaestecker *et al.* 2007) and, later, to identify genome-wide signatures of selection caused by the endoparasite *Pasteuria ramosa* (Orsini *et al.* 2012). The OM2 core spans approximately 40 years, sampled over 8 depths. A total of 68 distinct genotypes were hatched and established as clonal lines from this sedimentary archive. For the purpose of the current study the 8 sampled depths are considered (sub)populations, each spanning approximately 5 years.

*Core LR (Lake Ring, Denmark)* (55° 57' 51.83" N, 9° 35' 46.87" E). This core was obtained in 2004 from a shallow pond in Denmark. The pond was pristine until the late 1950s when agricultural runoff and sewage inflow from a nearby town initiated symptoms of severe eutrophication. The sewage inflow was diverted from the lake in the late 1970s, leading to the partial recovery of the lake from eutrophication starting from the 1980s. Hence, the

eutrophication history of the lake consists of four periods: pristine conditions (prior to 1950), severe eutrophication (1960-1970), a transition phase following the diversion of sewage inflow (after 1985) and a return to clear-water conditions (after 1999) (Michels 2008). To analyze changes in genetic diversity and composition through time of this lake we genotyped both dormant and hatched (sub)populations covering 50 years of history encompassing three lake phases: the eutrophic, transition and clear-water phase. The samples were grouped in four temporal equally-spaced (sub)populations, each representing a time period of 6 years to enable a fine-grained analysis. The dating of this sediment core was conducted using a classic radioisotope approach (Appleby 2001) in 2015. We analyzed 96 genotypes from hatched dormant eggs and 48 genotypes from unhatched dormant eggs.

*Spatial survey (Belgium).* This dataset comprises 19 populations of *D. magna* previously sampled by hatching dormant eggs from the surface sediment layers (the most recent 3 to 5 years) of 19 ponds in Belgium. Clonal lines were established for each genotype and used in a study to assess adaptive responses to three environmental stressors: fish predation, land use and parasite infection (Orsini *et al.* 2012). The sample sizes of these 19 populations range between 12 and 51 genetically distinct genotypes. They are used in this study, in addition to the (sub)populations isolated from the sediment cores, to identify the threshold sample size needed to represent genetic diversity in *D. magna* and to identify the combination of sample sizes and marker sets required to reliably estimate genetic diversity in the study species.

# *Genetic markers*

We quantified changes in genetic diversity over time within the three biological archives described in the previous section. As we focus on neutral genetic variation, our first action was to

202 identify a set of neutral loci among the ones used. A previous study on populations from the  
203 spatial survey, the OH and OM2 cores (Orsini *et al.* 2012) adopted an outlier approach  
204 (Beaumont 2005; Beaumont & Balding 2004) to identify loci putatively under selection for three  
205 environmental stressors known to induce evolutionary responses in *D. magna* (Orsini *et al.*  
206 2012). The populations from the spatial survey were sampled along three orthogonal gradients of  
207 selection represented by land use, fish predation, and parasite infection by the endoparasite  
208 *Pasteuria ramosa*. To identify outlier loci, populations diverging at only one stressor while  
209 identical with respect to the other two stressors were contrasted in multiple pairwise  
210 comparisons. (Orsini *et al.* 2012). Outlier loci identified in the spatial survey were then validated  
211 using temporal surveys. For these temporal surveys (sub)populations resurrected from different  
212 time periods along three sediment cores with known history of exposure to one of the three  
213 stressors investigated in the spatial survey, including the OH and OM2 cores in this study, were  
214 contrasted in an outlier analysis. Hence, for each stressor a spatio-temporal analysis of multiple  
215 pairwise population comparison was conducted. Only outliers found across multiple spatio-  
216 temporal pairwise comparisons were retained as putatively under selection. The set of neutral  
217 loci used here consisted of loci that were not associated with any of the environmental stressors  
218 previously analyzed. The OH core was also genotyped with SNP markers for this study using a  
219 SNP-chip (NimbleGen, Roche) developed to construct a high-density linkage map in *D. magna*  
220 (Routtu *et al.* 2014). The NimbleGen (Roche) array contained probes that interrogated 1,324  
221 SNPs, and included the SNPs previously used to genotype the OH and OM2 cores (Orsini *et al.*  
222 2012). After excluding loci with low amplification success (loci with more than 30% failings),  
223 950 SNPs were retained. A total of 873 SNPs were polymorphic in the samples of the OH core  
224 and were used for downstream analyses. Following Orsini *et al.* (2012), we performed a genome

scan analysis on the 873 SNPs using Fdist (Beaumont & Nichols 1996) implemented in Lositan (Antao *et al.* 2008; Beaumont & Nichols 1996) and contrasted the three time periods (B, M, T) in all possible pairwise combinations, identifying outlier loci putatively under selection for fish predation. This exercise allowed us to identify 840 neutral SNP loci, which we used for downstream analyses (Table S1).

The LR core was genotyped here for the first time at 45 microsatellite loci, representing a subset of the loci used for genotyping the OH and OM2 cores (Table S1). The core was subdivided in four (sub)populations encompassing 6-years each and covering a total time period of 50 years. From each (sub)population both hatched *D. magna* and unhatched dormant eggs were genotyped. To identify outlier loci putatively linked to eutrophication, we adopted the same approach of Orsini *et al.* (2012) and contrasted (sub)populations from the eutrophic, transition and clear water phases in pairwise combinations from both the hatched and unhatched (sub)populations. We retained 41 neutral loci, which we used for downstream analyses.

### *Change in genetic diversity over time*

To assess temporal stability in genetic diversity, we compared population genetic diversity indices among time periods [(sub)populations] within sediment cores. We quantified observed and expected heterozygosity ( $H_o$  and  $H_e$ ), and allelic richness (AR) using the diveRsity package in R (Keenan *et al.* 2013).

Partition of genetic diversity among (sub)populations within sediment cores was assessed with a two-level analysis of molecular variance (AMOVA) using Arlequin (Excoffier *et al.* 2005), on microsatellite and SNP markers separately. The two hierarchical levels were within and among

(sub)populations sampled along the sediment cores. This analysis was performed separately for the three cores.

#### *Comparing hatched and non-hatched populations*

If dormant stages are non-randomly resilient to hatching cues, the estimates of genetic diversity obtained on hatched (sub)populations may differ from the ones based on dormant egg banks. To assess whether this bias exists, we compared population genetic diversity and structure in hatched and unhatched *D. magna* (sub)populations from Lake Ring over a period of 50 years. Heterozygosity and allelic richness were quantified in both data sets. Moreover, the partition of genetic diversity (AMOVA, Excoffier *et al.* 2005) among the (sub)populations along the sediment core was assessed on both the hatched and unhatched (sub)populations using the hierarchical levels described above.

Changes in genetic structure based on individuals were assessed using STRUCTURE (Falush *et al.* 2003; Pritchard *et al.* 2000). After testing different parameter settings the following were used based on the stability of the MCMC parameters: 1,000,000 burn-in period, 100,000 MCMC iterations, uncorrelated loci and admixture model using population information as *prior*. Different prior of K were tested, ranging from 1 to 10 in triplicates. To estimate the number of clusters identified by STRUCTURE we used the Evanno method (Evanno *et al.* 2005) implemented in HARVESTER (Earl & vonHoldt 2012). To estimate whether the allelic profiles between the hatched and unhatched (sub)populations were comparable we studied the allelic composition of 6 microsatellite loci randomly chosen from the set used in this study. The comparison of allelic profiles was performed after standardizing the hatched (sub)populations to

the smallest sample size of the unhatched (sub)populations. The standardization was performed using the resampling with replacement approach described in the following section.

#### *Threshold sample size to capture genetic diversity changes in D. magna*

To assess the threshold sample size needed to obtain an unbiased estimate of genetic diversity changes in *D. magna* populations we performed a rarefaction analysis by drawing random subsamples with replacement (Luikart *et al.* 2010) from each (sub)population extracted from the three biological archives using the largest available markers set. At each random draw, 5 individuals were removed from the total set of individuals until a minimum sample size of 5 individuals was reached; additionally sample sizes smaller than 5 individuals were tested ( $N = 2, 3, 4$ ). This analysis was also conducted on the set of 19 populations from the spatial survey, which offer a large independent set of populations with, on average, larger sample sizes (Orsini *et al.* 2012). Rarefaction curves were obtained for the key population genetic indices:  $H_o$ ,  $H_e$ , and  $AR$ . These rarefaction curves allowed us to identify changes in key population genetic parameters as a function of sample size and to identify the threshold sample size minimally required to estimate genetic diversity in *D. magna* populations.

For species with limited genetic resources, the number of molecular markers available can be small. To assess the optimal combination of markers and sample sizes needed to estimate genetic diversity in our study species we performed a rarefaction analysis in which genetic diversity indices were calculated with different number of markers and sample sizes. For this analysis we performed random resampling of markers with replacement on the subsamples of the rarefaction analysis described above, effectively measuring population genetic parameters on all combinations of sample sizes and number of markers to a minimum sample size of 2 individuals

and 10 markers. This analysis was conducted on three random populations from the spatial survey that had sufficiently large sample sizes and on the hatched and unhatched (sub)populations of Lake Ring. We used a total of 70 microsatellites and 40 SNPs for the three populations from the spatial survey and 40 microsatellites for the (sub)populations from Lake Ring.

## Results

### *Change in genetic diversity over time*

The results from multiallelic (microsatellites) and biallelic (SNPs) markers were congruent, reflecting stable genetic diversity over time. All (sub)populations, with the exception of two in the OM2 core (D4 and D5 in the SNPs analysis), were in Hardy-Weinberg equilibrium. Heterozygosity and allelic richness within marker type were stable over time in all three biological archives (Fig 1A-C, Table S2).

The AMOVA analysis also showed congruent results for microsatellite and SNP markers. For all cores the largest fraction of molecular variance was at the within (sub)population level (Table 1), consistent with results of previous studies on *D. magna* (Orsini *et al.* 2012; Orsini *et al.* 2013c). In line with these previous results, the proportion of molecular variance among (sub)populations was small but significant. This result was observed for both multiallelic and biallelic markers.

### *Comparing hatched and non-hatched populations*

Heterozygosity did not differ significantly between the hatched and unhatched (sub)populations in the LR core (Fig 1A, LR) (t-test,  $P=0.54$ ). Allelic richness between the two sets of (sub)populations, calculated after standardizing the (sub)populations to the smallest sample size,

315 did not significantly differ (t-test,  $P=0.55$ ). The standardization was performed by randomly  
316 resampling the hatched (sub)populations with replacement to a sample size identical to the  
317 unhatched (sub)populations using the resampling with replacement approach described in the  
318 methods section.

319 In both hatched and unhatched populations the amount of molecular variance at the within and  
320 among (sub)population levels as quantified by the AMOVA was comparable. More specifically,  
321 a large proportion of the molecular variance was explained at within (sub)population level and a  
322 smaller yet significant proportion was explained at among (sub)populations level. This result  
323 reflects the one obtained for the other two sediment cores studied here (Table 1) and the results  
324 obtained in previous studies (Orsini *et al.* 2013a; Orsini *et al.* 2012). The STRUCTURE analysis  
325 identified comparably low genetic structure in the hatched and unhatched population sets (Fig 2).  
326 The Evanno method identified 7 clusters in the hatched (sub)populations and 6 in the unhatched  
327 (sub)populations (Table S3). The STRUCTURE plots corresponding to these clusters show that  
328 no obvious changes occurred over time in Lake Ring at neutral microsatellite loci. The allelic  
329 profiles between hatched and unhatched (sub)populations were congruent. Moreover, the alleles  
330 with high frequency are the same in the hatched and unhatched (sub)populations (Fig. S1).

### 332 *Threshold sample size to capture genetic diversity changes in D. magna*

333 This analysis aimed at identifying the optimal combination of sample size and number of  
334 markers to capture genetic diversity in our populations. Not surprisingly, this analysis showed  
335 that genetic diversity indices display different levels of sensitivity to the combinations of these  
336 key parameters. More specifically, for sample sizes larger than 5 individuals, estimates of  
337 heterozygosity were robust across the marker sets and sample sizes (Fig. 3 and Fig. S2).



Conversely, allelic richness was more sensitive to small sample sizes (Fig. 3 and Fig. S2) especially when small markers sets were genotyped (Fig.4 and Fig. S3). In summary, our analysis suggests that for heterozygosity and allelic richness a sample size of 5-10 individuals genotyped at 20 markers or more provides unbiased estimates of genetic diversity in our study species (Fig.4 and S3; Table S4).

## Discussion

### *Temporal stability of genetic diversity*

Temporal stability of genetic diversity is important because it determines the evolutionary potential of natural populations and their ability to persist in the face of environmental change. Studies of temporal changes conducted on a wide array of species (*e.g.* Alasaad *et al.* 2011; DeFaveri & Merila 2015; Goetze *et al.* 2015; Larsson *et al.* 2010; Tessier & Bernatchez 1999; Welch *et al.* 2012) report genetic stability and no reduction in genetic diversity over time. However, as most of these studies are limited in the number of years or generations studied or in the number of genetic markers used, the power to detect changes in genetic diversity is often limited. Here, we use layered dormant egg banks to reconstruct genetic diversity in natural populations over periods spanning several decades (20 to 50 years) in an organism with a cyclic parthenogenetic life cycle and a generation time of approximately 14 days. In addition, we assess genetic diversity through time using multi- and bi-allelic markers.

Our results show no apparent genetic erosion in *D. magna* populations with time (from old to recent populations), even following a period of strong selection pressure. In the time periods studied here, considering the short generation time of the waterflea, strong differential mortality

360 or genetic drift can occur. Lack of genetic erosion with time suggests that genetic drift and  
361 selection have no detectable impact on neutral genetic diversity in the populations and over the  
362 time scales studied. Our results show no reduction in genetic diversity as the egg banks age  
363 (from recent to old populations), at least over the multi-decadal time covered by our study. This  
364 result shows that there is no reduction in genetic diversity because of differential mortality of old  
365 dormant eggs, and hence, indicates that dormant egg banks can be reliably used to study genetic  
366 changes in natural populations. Genetic stability in a zooplankter such as *Daphnia* is striking  
367 given that there are many reports of strong shifts in clonal composition during the growing  
368 season in active *Daphnia* populations (De Meester *et al.* 2006; Hebert 1974; Lynch 1984) and of  
369 clonal erosion as the growing season progresses (Vanoverbeke & De Meester 2010). Moreover,  
370 while the census population size of *Daphnia* populations is typically very large, several studies  
371 have estimated the effective population size ( $N_e$ ) of natural populations of this cyclical  
372 parthenogen to be rather small (Hamrova *et al.* 2011; Orsini *et al.* 2013a; Orsini *et al.* 2012) with  
373 even more reduced  $N_e$  in populations with higher turnover (Walser & Haag 2012). Small  $N_e$  can  
374 potentially lead to genetic drift (Hartl & Clark 2007). If genetic drift occurred, it would be visible  
375 as a significant allele frequency difference between years (e.g. Charlier *et al.* 2012) and reduced  
376 allele richness and heterozygosity over time. We do not observe these patterns and observe  
377 instead stability in neutral genetic variation, even though earlier findings show clear signature of  
378 selection and hence reduced diversity in loci under selection or linked to genes under selection in  
379 two of the biological archives studied here (Orsini *et al.* 2012). This suggests that whereas  
380 environmental selection pressure can impact target loci or genomic regions, neutral genetic  
381 diversity, and possibly effective population size, is conserved over time even in presence of  
382 strong selection pressure.

Genetic stability is likely maintained by the buffering effect of the resting egg bank, a concept put forward in the late 1980's as a mechanism that zooplankton adopts to survive environmental hardship (Hairston *et al.* 1999). Similarly, in a community context, the buffering effect of the dormant egg bank has been used to explain species diversity (storage effect, Caceres 1997; Chesson & Warner 1981). A simulation study demonstrated that the buffering effect of dormant individuals mitigates if not eliminates the impact of chaotic environmental dynamics (Lalonde & Roitberg 2006), helping species with dormant stages to better cope with chaotic environments (environments that change unpredictably). The temporal stability in neutral genetic diversity we observe here, even in presence of strong selection pressure as opposed to a clear signature of selection on specific loci or regions of the genome, suggest that the dormant egg bank may act as buffer of genetic diversity. This hypothesis is supported by the knowledge that strong changes in the environment occurred in all three habitats and the observation that in two of the three studied biological archives earlier resurrection ecology studies have revealed rapid and adaptive evolutionary responses of the *Daphnia* population to environmental change (Cousyn *et al.* 2001; Decaestecker *et al.* 2007; Michels 2008; Pauwels *et al.* 2010 ; Stoks *et al.* 2016). Furthermore, a population genomic study of the same two biological archives identified signatures of selection and shifts in genetic composition in selected regions of the genome in response to the same environmental changes (Orsini *et al.* 2012). Hence, the absence of genetic erosion at neutral markers was observed whilst significant change in genotypic trait values and markers under selection was documented in response to environmental change.

The study of temporal genetic stability in species that produce dormant stages is in its infancy, our study being the first to formally address this issue using three sedimentary archives. The patterns we observed are repeatable across archives exposed to different environmental

stress providing support that our observations are not population- specific. However, the absence of similar studies in other taxa, or even other *Daphnia* species, does not allow us to conclude whether the trends observed in this study can be generalized. Further studies on temporal genetic stability in species producing dormant stages will be of critical importance to conclude whether the patterns we observed in *D. magna* can be extended to other species or taxa.

We show that genetic diversity changes and allelic composition through time in one of the sedimentary archives are comparable between hatched and unhatched (sub)populations, suggesting that there is no bias in genetic diversity and composition of the hatched (sub)populations as compared to the dormant ones. These results indicate that the use of unhatched dormant stages could be embraced in allochronic studies without introducing bias in the study of evolutionary responses to selection pressure. However, we acknowledge that a comparative analysis of hatched and unhatched (sub)populations should be conducted on additional archives for the same species and possibly for other species before we can confidently conclude that the patterns observed for Lake Ring are common to other populations and/or species. If the patterns observed in the sedimentary archive of Lake Ring are observed in other archives, this critically demonstrates that dormant stages can be used in place of hatched individuals when decreasing hatching success hampers the application of resurrection ecology. Limited hatching success with increasing age of the dormant eggs is an acknowledged limitation of resurrection ecology and one of the main reasons why most studies are limited to the recent past (but see Frisch *et al.* 2014 for an exceptional study).

We show that when a relatively high number of molecular markers (from 41 to 840) is employed, a sample size of five to ten distinct genotypes is sufficient to obtain good estimates of genetic diversity. We also show that for smaller markers sets (20 loci) a sample size of 10-15

individuals reliably estimates population genetic diversity. These finding have important implication for resurrection ecology studies in which the number of hatchlings tends to decrease with the age of the sediment. It is also relevant for studies directly measuring genetic diversity of the egg bank, when limited number of dormant eggs can be retrieved. The amount of sediment that can be retrieved from natural habitats is generally not limiting in temperate regions but can be logistically challenging when sampling polar or high altitude lakes. In these circumstances sediment cores of small diameter (e.g. 6 cm or less) are generally used, and hence limited material can be retrieved per layer of sediment. Our findings have also important implications for species that have limited genetic resources. Our results suggest that a limited number of individuals can reliably represent the genetic pool of temporal (sub)populations and that a combination of 15 individuals genotyped at 20 markers reliably estimates genetic diversity in space and time. Whether this applies to other species remains to be seen. If these results will be confirmed in other species producing dormant stages, evolutionary and population genetic studies can extend beyond the decadal time scale analyzed here and potentially enable paleogenomic studies over centuries and millennia.

#### *The use of biological archives in evolutionary applications*

Temporally spaced DNA samples offer a unique opportunity to study genetic changes in response to changes in the environment. By comparing the genetic composition of a population before and after a well-documented environmental change, it is possible to track changes in allele frequencies for a retrospective ‘real time’ assessment of genetic impacts. To date, very few studies were able to track genetic changes in natural populations associated with environmental change using genetic markers (Frisch *et al.* 2014; Larsson *et al.* 2010; Welch *et al.* 2012;

Yashina *et al.* 2012). Genomic tools applied to dormant eggs open up possibilities for reconstructing the evolutionary history of natural populations over hundreds of years (Frisch *et al.* 2014; Mergeay *et al.* 2007; Orsini *et al.* 2013b; Orsini *et al.* 2012). With the advent of third generation sequencing technologies, which enables the sequencing of genomes and transcriptomes from just a few cells (Dey *et al.* 2015), more technical limitations are being lifted, enabling the application of ‘omics’ technologies to limited and degraded material, unthinkable until few years ago. The power of performing ‘omics’ studies on dormant stages is further amplified by the fact that for *D. magna* and possibly other crustaceans the sample size required to represent genetic diversity of temporal populations is small, being in the order of 10 distinct genotypes, as our rarefaction analysis demonstrates. Similar studies in other taxa will allow us to confirm whether such small sample sizes can be applied widely in population genetics of species with dormant stages.

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## References

- Alasaad S, Oleaga A, Casais R, *et al.* (2011) Temporal stability in the genetic structure of *Sarcoptes scabiei* under the host-taxon law: empirical evidences from wildlife-derived *Sarcoptes* mite in Asturias, Spain. *Parasit Vectors* **4**, 151.
- Altshuler I, Demiri B, Xu S, *et al.* (2011) An integrated multi-disciplinary approach for studying multiple stressors in freshwater ecosystems: *Daphnia* as a model organism. *Integr Comp Biol* **51**, 623-633.
- Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics* **9**, 323.
- Appleby PG (2001) *Chronostratigraphic techniques in recent sediments* Kluwer Academic Publisher, The Netherlands.
- Appleby PG, Nolan PJ, Gifford DW, *et al.* (1986) PB-210 dating by low background gamma-counting. *Hydrobiologia* **143**, 21-27.
- Barrick JE, Yu DS, Yoon SH, *et al.* (2009) Genome evolution and adaptation in a long-term experiment with *Escherichia coli*. *Nature* **461**, 1243-1247.
- Beaumont MA (2005) Adaptation and speciation: what can Fst tell us? *Trends Ecology and Evolution* **20**, 435-440.
- Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Mol Ecol* **13**, 969-980.
- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society B* **363**, 1619-1626.
- Bidle KD, Lee SH, Marchant DR, Falkowski PG (2007) Fossil genes and microbes in the oldest ice on Earth. *Proceedings National Academy Science USA* **104**, 13455-13460.
- Blount ZD, Barrick JE, Davidson CJ, Lenski RE (2012) Genomic analysis of a key innovation in an experimental *Escherichia coli* population. *Nature* **489**, 513-518.
- Caceres CE (1997) Temporal variation, dormancy, and coexistence: a field test of the storage effect. *Proc Natl Acad Sci U S A* **94**, 9171-9175.
- Charlier J, Laikre L, Ryman N (2012) Genetic monitoring reveals temporal stability over 30 years in a small, lake-resident brown trout population. *Heredity* **109**, 246-253.
- Chesson PL, Warner RR (1981) Environmental Variability Promotes Coexistence in Lottery Competitive Systems *American Naturalist* **117**, 923-943.
- Cousyn C, De Meester L, Colbourne JK, *et al.* (2001) Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *PNAS* **98**, 6256-6260.
- De Meester L, Vanoverbeke J, De Gelas K, Ortells R, Spaak P (2006) Genetic structure of cyclic parthenogenetic zooplankton populations—A conceptual framework. *Arch. Hydrobiol* **167**, 217-244.
- Decaestecker E, Gaba S, Raeymaekers J, *et al.* (2007) Host-parasite Red Queen dynamics archived in pond sediment. *Nature* **450**, 870-874.
- DeFaveri J, Merila J (2015) Temporal stability of genetic variability and differentiation in the three-spined stickleback (*Gasterosteus aculeatus*). *PLoS One* **10**, e0123891.
- Dey SS, Kester L, Spanjaard B, Bienko M, van Oudenaarden A (2015) Integrated genome and transcriptome sequencing of the same cell. *Nat Biotechnol* **33**, 285-289.

- 517 Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for  
518 visualizing STRUCTURE output and implementing the Evanno method. *Conservation*  
519 *Genetics Resources* **4**, 359-361
- 520 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the  
521 software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-2620.
- 522 Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for  
523 population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47-50.
- 524 Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus  
525 genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567-1587.
- 526 Frisch D, Morton PK, Chowdhury PR, *et al.* (2014) A millennial-scale chronicle of evolutionary  
527 responses to cultural eutrophication in *Daphnia*. *Ecol Lett* **17**, 360-368.
- 528 Fukami T, Wardle DA (2005) Long-term ecological dynamics: reciprocal insights from natural  
529 and anthropogenic gradients. *Proceedings of the Royal Society B-Biological Sciences*  
530 **272**, 2105-2115.
- 531 Goetze E, Andrews KR, Peijnenburg KTCA, Portner E, Norton EL (2015) Temporal Stability of  
532 Genetic Structure in a Mesopelagic Copepod. *PLoS One* **10**.
- 533 Grant PR, Grant BR (2002) Unpredictable evolution in a 30- year study of Darwin's finches.  
534 *Science* **296**, 707-711.
- 535 Hairston JNG, Lampert W, Caceres CE, *et al.* (1999) Rapid evolution revealed by dormant eggs.  
536 *Nature* **401**, 446.
- 537 Hamrova E, Mergeay J, Petrusek A (2011) Strong differences in the clonal variation of two  
538 *Daphnia* species from mountain lakes affected by overwintering strategy. *Bmc*  
539 *Evolutionary Biology* **11**.
- 540 Härnström K, Ellegaard M, Andersson TJ, Godhe A (2011) Hundred years of genetic structure  
541 in a sediment revived diatom population. *Proceedings National Academy Science USA*  
542 **108**, 4252-4257.
- 543 Hartl DL, Clark AG (2007) *Principles of Population Genetics, Fourth Edition* Sinauer and  
544 Associates, Sunderland, MA.
- 545 Hebert PDN (1974) Ecological differences between genotypes in a natural population of *Daphnia*  
546 *magna*. *Heredity* **33**, 327-337.
- 547 Jankowski T, Straile D (2003) A comparison of egg-bank and long-term plankton dynamics of  
548 two *Daphnia* species, *D-hyalina* and *D-galeata*: Potentials and limits of reconstruction.  
549 *Limnology and Oceanography* **48**, 1948-1955.
- 550 Kawecki TJ, Lenski RE, Ebert D, *et al.* (2012) Experimental evolution. *Trends in Ecology and*  
551 *Evolution* **27**, 547-560.
- 552 Keenan K, McGinnity P, Cross TF, Crozier WW, Prodoh PA (2013) diveRsity: An R package for  
553 the estimation and exploration of population genetics parameters and their associated  
554 errors. *Methods in Ecology and Evolution* **4**, 782-788.
- 555 Kerfoot WC, Robbins JA, Weider LJ (1999) A new approach to historical reconstruction:  
556 Combining descriptive and experimental paleolimnology. *Limnology and Oceanography*  
557 **44**, 1232-1247.
- 558 Lalonde RG, Roitberg BD (2006) Chaotic dynamics can select for long-term dormancy.  
559 *American Naturalist* **168**, 127-131.
- 560 Larsson LC, Laikre L, Andre C, Dahlgren TG, Ryman N (2010) Temporally stable genetic  
561 structure of heavily exploited Atlantic herring (*Clupea harengus*) in Swedish waters.  
562 *Heredity (Edinb)* **104**, 40-51.



- Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW (2010) Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conservation Genetics* **11**, 355-373.
- Lynch M (1984) The genetic structure of a cyclical parthenogen. *Evolution* **38**, 186-203.
- Marcus NH (1990) Calanoid copepod, cladoceran, and rotifer-eggs in sea-bottom sediments of northern Californian coastal waters: identification, occurrence and hatching. *Marine Biology* **105**, 413-418.
- Mergeay J, Vanoverbeke J, Verschuren D, De Meester L (2007) Extinction, recolonization, and dispersal trough time in a planktonic crustacean *Ecology* **88**, 3032-3043
- Merila J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications* **7**, 1-14.
- Michels H (2008) *Micro-evolutionary response of Daphnia magna to changes in biotic stress associated with habitat degradation and restoration of a shallow lake* Doctoral University of Leuven.
- Miner BE, De Meester L, Pfrender ME, Lampert W, Hairston NG (2012) Linking genes to communities and ecosystems: Daphnia as an ecogenomic model. *Proceedings of the Royal Society B-Biological Sciences* **279**, 1873-1882.
- Onbe T (1978) Sugar flotation method for sorting the resting eggs of marine cladocerans and copepods from sea-bottom sediment. *Bulletin Japanese Society Scientific Fisheries* **44**, 1411.
- Orsini L, Mergeay J, Vanoverbeke J, De Meester L (2013a) The role of selection in driving landscape genomic structure of the waterflea *Daphnia magna*. *Molecular Ecology* **22**.
- Orsini L, Schwenk K, De Meester L, *et al.* (2013b) The evolutionary time machine: using dormant propagules to forecast how populations can adapt to changing environments. *Trends in Ecology and Evolution* **28**, 274-282.
- Orsini L, Spanier KI, De Meester L (2012) Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. *Molecular Ecology* **21**, 2160-2175.
- Orsini L, Vanoverbeke J, Swillen I, Mergeay JDM, L. (2013c) Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology* **22**, 5983-5999.
- Pauwels K, Stoks R, De Meester L (2010 ) Enhanced anti-predator defence in the presence of food stress in the water flea *Daphnia magna*. *Functional Ecology* **24**, 322-329.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.
- Reznick DN, Shaw FH, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**, 1934-1937.
- Routtu J, Hall MD, Albrecht B, *et al.* (2014) An SNP-based second-generation genetic map of *Daphnia magna* and its application to QTL analysis of phenotypic traits. *BMC Genomics* **15**, 1033.
- Stoks R, Govaert L, Pauwels K, Jansen B, De Meester L (2016) Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. *Ecology Letters* **10.1111/ele.12551**.

Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). *Molecular Ecology* **8**, 169-179.

Vanoverbeke J, De Meester L (2010) Clonal erosion and genetic drift in cyclical parthenogens - the interplay between neutral and selective processes. *Journal of Evolutionary Biology* **23**, 997-1012.

Walser B, Haag CR (2012) Strong intraspecific variation in genetic diversity and genetic differentiation in *Daphnia magna*: the effects of population turnover and population size. *Molecular Ecology* **21**, 851-861.

Weider LJ, Pijanowska J (1993) Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos* **67**, 385-392.

Welch AJ, Wiley AE, James HF, *et al.* (2012) Ancient DNA Reveals Genetic Stability Despite Demographic Decline: 3,000 Years of Population History in the Endemic Hawaiian Petrel. *Molecular Biology and Evolution* **29**, 3729-3740.

Yashina S, Gubinb S, Maksimovich S, *et al.* (2012) Regeneration of whole fertile plants from 30,000-y-old fruit tissue buried in Siberian permafrost. *Proceedings National Academy Science USA* **109**, 4008-4013.

**Data accessibility**

SNP and microsatellites genotypes for populations from the spatial survey are deposited in the DRYAD databank at the following entries: <http://dx.doi.org/10.5061/dryad.384rr593.2/2.2> and <http://dx.doi.org/10.5061/dryad.384rr593.2/7.2>.

Microsatellites genotypes for the OH core are deposited in the DRYAD databank at the following entry: <http://dx.doi.org/10.5061/dryad.384rr593.2/10.2>.

SNP genotypes for the OH core and microsatellite genotypes for LR, hatched and unhatched, are deposited in the DRYAD databank at: doi:10.5061/dryad.p1k64

SNP and microsatellites genotypes for the OM2 core are deposited in the DRYAD databank at the following entries: <http://dx.doi.org/10.5061/dryad.384rr593.2/3.2> and <http://dx.doi.org/10.5061/dryad.384rr593.2/8.2>.

**Authors' contribution:**

638 LO and LDM conceived the study; HM and MCC generated and analyzed the data on Lake Ring;  
639 AC performed the rarefaction analysis; KIS contributed to data analysis; KWT and MEP  
640 generated SNP chip data for the OH core; LO generated and analyzed the data and wrote the  
641 paper. All authors contributed to the final editing of the paper.

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**Table 1. AMOVA analysis.** Partitioning of genetic variance within and among (sub)populations within cores estimated with an Analysis of Molecular Variance. The two hierarchical levels used in the analysis are (1) among the (sub)populations along each sediment core and (2) within (sub)population. Statistically significant values (\*,  $P < 0.001$ ) are based on permutation tests (10,000 permutations). For the LR core the AMOVA results are shown for both for the hatched and unhatched populations at microsatellite loci; for the other cores results are shown for microsatellite and SNP markers.

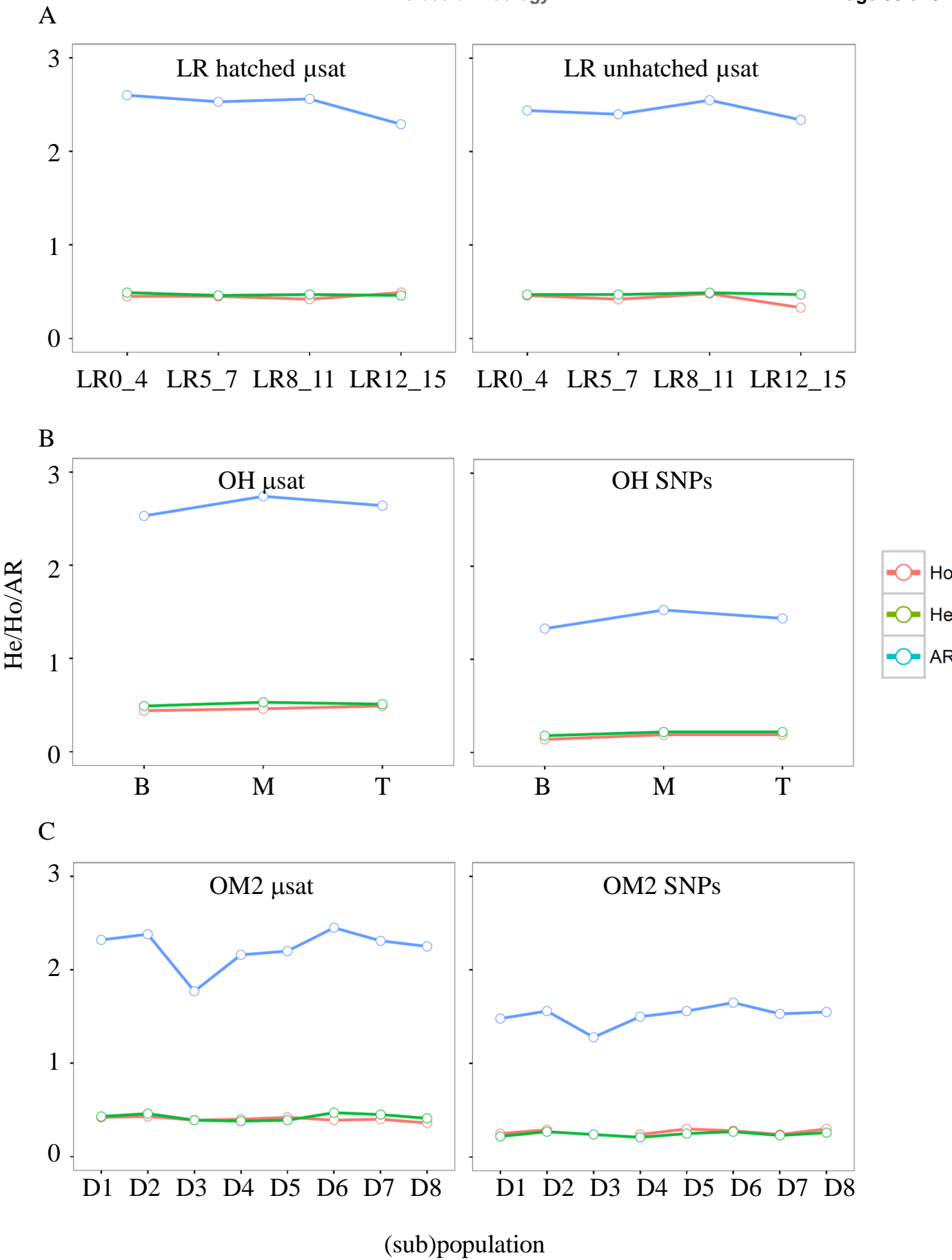
	Among (sub)populations	Within (sub)populations
<i>OH core</i>		
Neutral $\mu$ sat	4.82*	95.18*
Neutral SNPs	1.04*	98.96*
<i>OM2 core</i>		
Neutral $\mu$ sat	2.94*	97.06*
Neutral SNPs	1.84*	98.16*
<i>LR core</i>		
Neutral $\mu$ sat (Hatched)	1.08*	98.92*
Neutral $\mu$ sat (Unhatched)	1.75*	98.25*

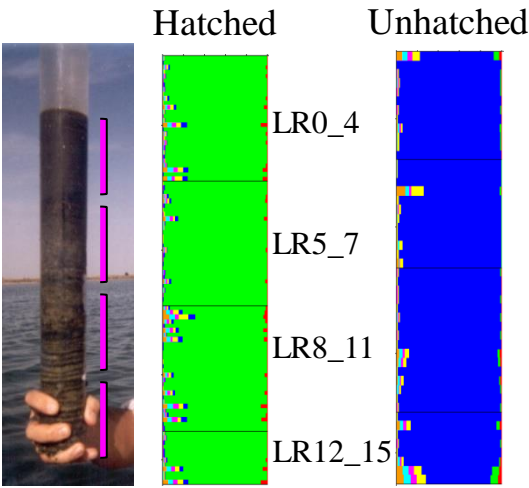
**Figure 1. Genetic diversity indices.** Variation at genetic diversity indices in the three sedimental archives. (A) Hatched and unhatched (sub)populations from Lake Ring genotyped at microsatellite markers. Populations LR12\_15, LR8\_11, LR5\_7, LR0\_4 represent different (sub)populations from old to recent. (B) (sub)populations from Oud Heverlee (OH) and (C) Oude Meren (OM2) ponds genotyped at microsatellite and SNP markers displayed from old to recent as follows: B=bottom, M=median, T=top for OH; and D1-8 = depth 1 to 8 for OM2. Observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), and allelic richness (AR) are shown.

**Figure 2. STRUCTURE analysis.** Population genetic structure changes are shown for hatched and unhatched populations of LR (Lake Ring). The color code for the alleles is randomly generated, hence identical colors may represent different alleles in different runs. Horizontal lines define (sub)populations, each spanning 6 years.

**Figure 3. Rarefaction curves for sample sizes.** Rarefaction curves are shown for heterozygosity ( $H_o$  and  $H_e$ ) and allelic richness (AR) in (sub)populations from (A) Lake Ring, for hatched and unhatched populations, (B) Oude Heverlee (OH core), and (C) Oude Meren (OM2 core). Samples from LR were genotyped at microsatellite loci whereas samples from the other two cores were genotyped at microsatellite and SNP markers. The rarefaction curves were obtained by randomly resampling sample size with replacement to a minimum sample size of two individuals. The rarefaction curves for the populations from the spatial survey confirming the patterns observed in the temporal (sub)populations are shown in Figure S2.

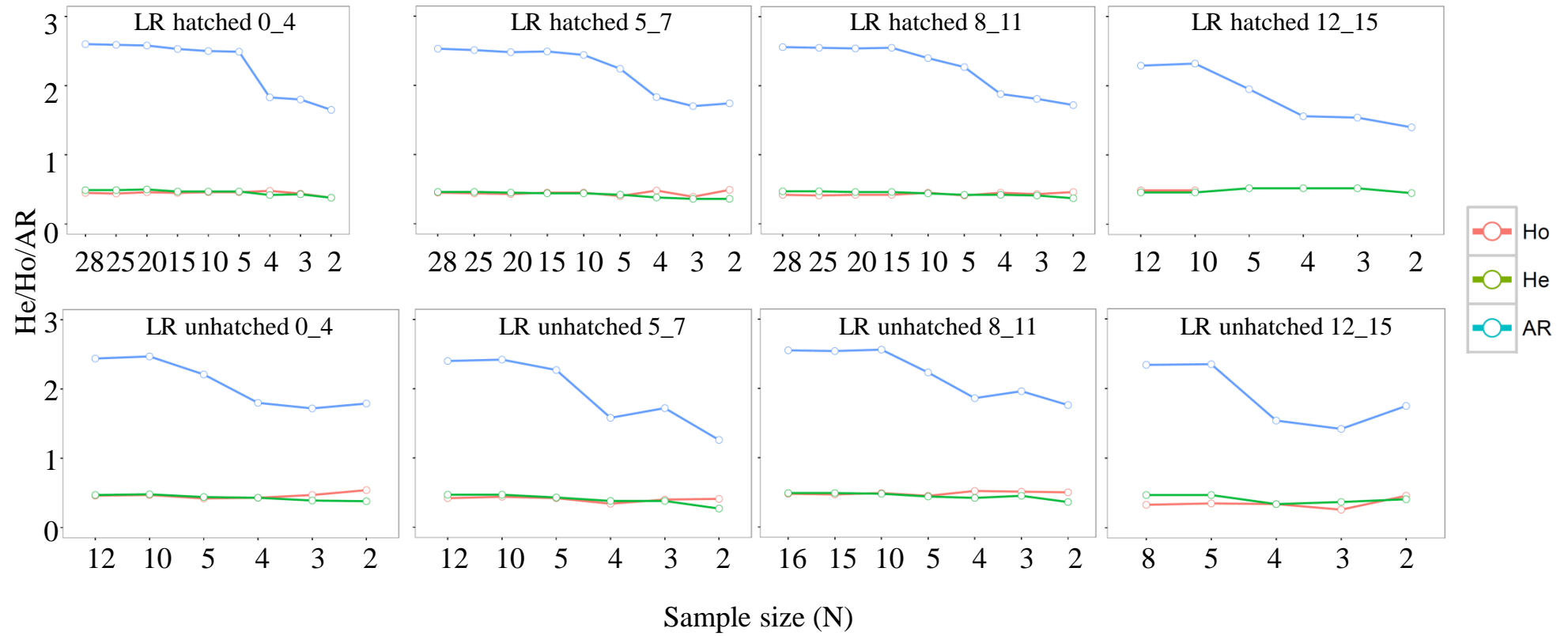
**Figure 4. Rarefaction curves for markers sets.** Rarefaction curves are shown for heterozygosity ( $H_o$  and  $H_e$ ) and allelic richness (AR) for three populations from the spatial survey genotyped at microsatellites (A) and SNPs (B). The different markers sets, randomly resampled with replacement to a minimum number of 10 markers, were tested on rarefied sample sizes to a minimum sample size of two individuals. These sample sizes are the rarefied subsamples in Fig S2. The rarefaction curves calculated for the (sub)populations from Lake Ring are shown in Figure S3.

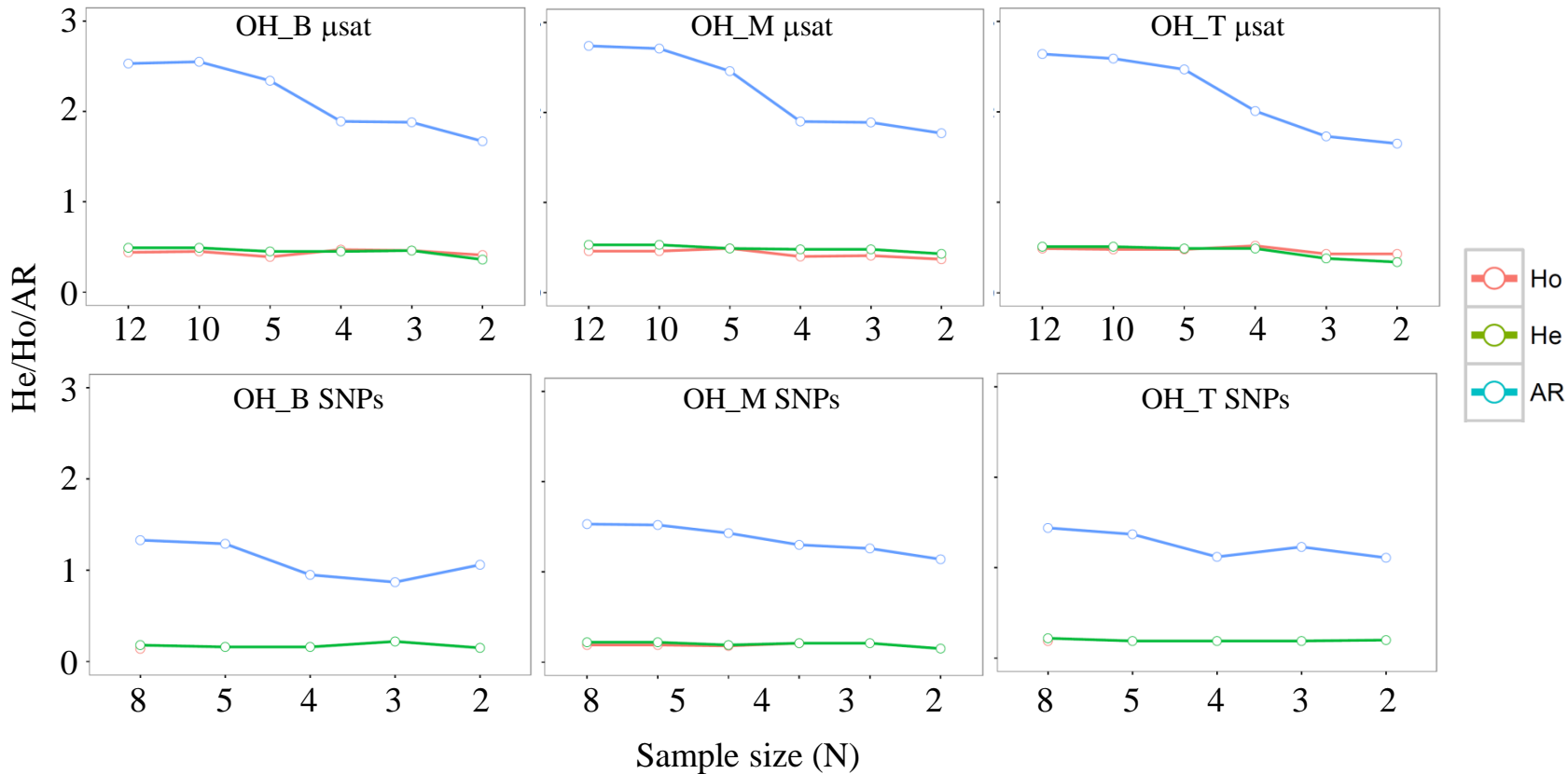






A





C

